Department of Health and Social Security

Report on Health and Social Subjects

22

THE COLLECTION AND STORAGE OF HUMAN MILK

Report of a Working Party on Human Milk Banks, Panel on Child Nutrition
Committee on Medical Aspects of Food Policy

s Stationery Office

Department of Health and Social Security

Report on Health and Social Subjects

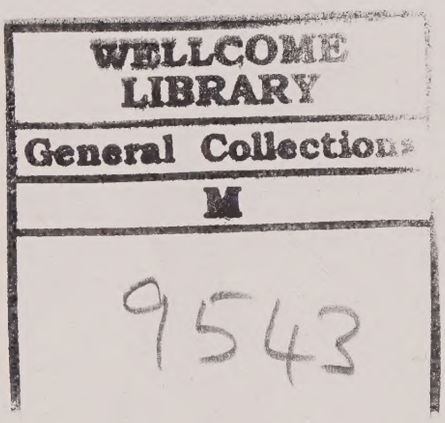
22

THE COLLECTION AND STORAGE OF HUMAN MILK

Report of a Working Party on Human Milk Banks, Panel on Child Nutrition
Committee on Medical Aspects of Food Policy

London
Her Majesty's Stationery Office

First published 1981



ISBN 0 11 320765 4

Preface

Human milk banks are known to have been in existence for the past half century, and were set up to provide human milk for babies who could not otherwise have obtained it. With the increasing refinement of artificial feeds prepared from cows' milk, appreciation of the value of human milk became temporarily under-rated and, over the past quarter of a century, many milk banks were closed. In the last decade, however, research has emphasized the unique nutritional and protective qualities of both human colostrum and milk, and the demand for donated human milk for small or sick babies, whose mothers cannot themselves provide enough for their infants, has increased.

In this technological age, standards for collecting, treating, storing and distributing human milk are expected to be high. Guidelines have been sought from the Health Departments by many professional staff in hospitals when proposals have been made to set up human milk banks. It is for Health Authorities locally to decide whether to set up human milk banks in the light of their particular circumstances. Established banks function successfully in many different ways and, in the present state of knowledge, a single correct procedure cannot be advocated. Nevertheless, it is essential to ensure the microbiological safety of any milk given to young babies as well as to retain as completely as possible the immunological and nutritional properties of the milk. The means by which a balance between these two functions of a milk bank can be achieved are discussed in this report of a Working Party set up under the aegis of the Panel on Child Nutrition of the Committee on Medical Aspects of Food Policy.

We are grateful to the Chairman, Professor June Lloyd, and to all the other members of the Working Party, who gave freely of their time and expertise in three meetings held between September 1979 and March 1980 and in correspondence by which much of the work was completed. We are also indebted to members of the Standing Panel on Hazards from Microbial Contamination of Food for their helpful comments on the microbiological aspects of the draft report.

H Yellowlees
Chairman,
Committee on Medical Aspects of Food Policy

Committee on Medical Aspects of Food Policy

Working Party on Human Milk Banks

Membership

Chairman:

Professor J K Lloyd	Department of Child Health, St George's Hospital Medical School, London

Members:

Mrs K Banks[1] (until February 1980)	Sorrento Hospital, Mosely, Birmingham
Dr J D Baum	Department of Paediatrics, John Radcliffe Hospital, Oxford
Dr S J Darke	Department of Health and Social Security, London
Dr D P Davies[2]	Department of Child Health, Leicester Royal infirmary, Leicester
Miss D E M Francis	The Hospitals for Sick Children, Great Ormond Street, London
Miss S Goodwin[3]	Health Visitors Association, London
Dr M I Gurr	National Institute of Research in Dairying, Shinfield, Reading, Berkshire
Dr V Miller[2]	Booth Hall Children's Hospital, Manchester

Dr C J Mitchell	Department of Pathology, Leicester Royal Infirmary, Leicester
Professor J W G Porter	National Institute for Research in Dairying, Shinfield, Reading, Berkshire
Mrs E Proctor[1] (from March 1980)	Sorrento Hospital, Mosely, Birmingham
Miss J Saunders[1]	Southmead Hospital, Westbury-on-Trym, Bristol
Mrs P Williams[4]	Southmead Hospital, Westbury-on-Trym, Bristol

Assessors:

Dr R Alderslade	Department of Health and Social Security, London
Miss A E King	Department of Health and Social Security, London
Miss B Weller	Department of Health and Social Security, London

Observers

Dr F B Davidson	Scottish Home and Health Department, Edinburgh
Dr F M Richards	Welsh Office, Cardiff

Secretariat:

Dr S R Fine	Department of Health and Social Security, London
Mr D K Smith	Department of Health and Social Security, London

(1) Nominated by the Royal College of Midwives
(2) Nominated by the British Paediatric Association
(3) Nominated by the Health Visitors Association
(4) Nominated by the British Dietetic Association

Contents

1. General Introduction

1.1 With the growing awareness of the importance of human milk since the publication of the report 'Present-Day Practice in Infant Feeding' (Department of Health and Social Security, 1974), milk banks are being used in many parts of the country to provide milk for babies whose mothers cannot breast-feed. In response to requests from the paediatric departments of many hospitals, a Working Party was set up to define operational guidelines so that the techniques used for collecting and storing donor milk are of optimal safety and efficiency.

1.2 The Working Party on Human Milk Banks under the aegis of the Panel on Child Nutrition of the Committee on Medical Aspects of Food Policy met for the first time in September 1979, with Professor June Lloyd as chairman. Members of the Working Party represented medical, nursing, dietetic and scientific expertise in the field.

1.3 The terms of reference were 'to advise about setting up Human Milk Banks and to make recommendations'.

1.4 The unique nutritional and protective (anti-microbial) properties of human milk are now well recognized and will not be described in this report. Published information may be obtained from many sources, including reports on the Composition of Mature Human Milk (Department of Health and Social Security, 1977), on Artificial Feeds for the Young Infant (Department of Health and Social Security, 1980a) and on Present Day Practice in Infant Feeding: 1980 (Department of Health and Social Security, 1980b).

1.5 This report discusses the case for human milk banks, the microbiological implications for safety in collection, storage and treatment of milk, the nutritional effects of the various procedures used, and the need for maintaining microbiological safety with retention of the maximum nutritional and anti-microbial characteristics of human milk. A section on the organizational aspects of banking donor milk gives detailed recommendations, and a separate section discusses procedure when the milk is provided by the mother for the use of her own baby. Appendices cover the practical microbiology involved in establishing that the criteria for microbiological safety are satisfied. A list is also appended of some established milk banks from which further information can be obtained.

2. The case for human milk banks

2.1 Provision for babies with special needs

2.1.1 Human milk is desirable for all babies but there are some, both in hospital and at home, for whom special efforts should be made to supply human milk because of its unique nutritional and immunological properties. These babies may be those who are pre-term, small or sick, those who are recovering in early infancy from surgery, those in whom there is a family history of atopy or who are otherwise at risk of cows' milk intolerance and those who are unable for short periods to feed from their mothers.

2.1.2 Ideally, the mothers of such babies could be expected to express their milk for feeding to their own babies. Although some mothers are successful in sustaining lactation and achieving this ideal, at present many seem to be unable to do so, and for their babies, human milk may be collected from other mothers who are producing milk in excess of the requirements of their own babies. Mothers who are able to donate their excess milk may be either in hospital or at home and it is for the purpose of collecting, treating, storing and distributing their surplus and valuable milk that human milk banks have been organized.

2.1.3 In past centuries wet nursing provided human milk for a baby whose mother did not breast-feed. No alternative was available for the survival of the baby but with the advent of artificial feeds (usually made from cows' milk) the practice of wet nursing lapsed. Nevertheless in many countries a demand continued for human milk, particularly for pre-term infants. Thus human milk banking has been carried out on a fairly large scale in Helsinki for over fifty years; a chain of grocery stores is used to collect the milk samples for delivery to the hospital milk bank and to distribute clean bottles to the mothers (Siimes and Hallman, 1978). Almost forty years ago recommended standards were drawn up for the operation of mothers' milk bureaux based on several years of experience of human milk banking in the United States (American Academy of Pediatrics, 1943).

2.1.4 In the United Kingdom from the 1930s onwards many maternity hospitals developed their own milk banks for the benefit of their infant patients. Additionally in a number of larger hospitals milk banks were set up which were able to provide milk for other hospital units over a wide area. When breast-feeding declined in popularity in the 1960s many, but not all, hospital milk banks closed down. In recent years, the reawakening of interest in infant nutrition and the better understanding of the biochemical, nutritional and immunological properties of human milk, especially in

relation to the needs of low birthweight babies, have encouraged the re-emergence of many more human milk banks. In 1978 an informal meeting in Oxford on Human Milk Banking Practices was attended by representatives from no fewer than 30 milk banks in the United Kingdom (Baum, 1979a).

2.2 Composition of milk in human milk banks

2.2.1 Lactating mothers who wish to donate some of their milk to hospital milk banks can collect the milk in one of two ways, or both methods may be used. Milk may either be expressed from the breast by hand or by breast-pump into a container — 'expressed breast milk' — or the milk which drips from the non-feeding breast during a feed may be collected into shells — 'drip breast milk'. Milk collected by these methods is often referred to as EBM and DBM respectively but in order to avoid possible confusion, these abbreviations will not be used in this report.

2.2.2 Expressed breast milk and drip breast milk differ in their composition. Changes in composition also occur in both types of milk in relation to the total duration of lactation. For these reasons milk collected by different hospital milk banks may vary considerably in composition and an understanding of the variation is important in order to be able to assess the nutritional value of the milk fed to babies.

2.2.3 *Changes in composition with method of collection.* Table 2.1 shows the concentration of some of the nutrients in pooled samples of expressed human milk from 96 mothers at 4-6 weeks post partum from 5 different areas of Great Britain (Department of Health and Social Security, 1977) and in pooled 48-hour collections of drip milk from up to 27 donors in the second to fourth month of lactation (Gibbs, Fisher, Bhattacharya, Goddard and Baum, 1977). The difference in the fat content is most striking; drip breast milk contains about half the amount of fat present in expressed breast milk, and thus resembles the milk taken by the breast-feeding baby at the beginning of a feed — 'fore milk'. As fat provides the chief source of energy in milk, drip breast milk therefore provides less energy than expressed breast milk which, in the Department of Health and Social Security analysis, was a mixture of fore milk and hind milk, because the whole of the milk content of one breast was expressed and collected. The concentrations of protein, lactose and the major minerals are not significantly different in drip and expressed milk and neither are the amounts of secretory immunoglobulin A (IgA) and lysozyme (Gibbs, Fisher, Bhattacharya, Goddard and Baum, 1977).

Table 2.1 *Average composition of human pooled expressed milk and drip milk*

		Expressed milk (1)	Drip milk (2)
Energy	Kcal/1	700	480
	MJ/1	2.9	2.0
Protein	g/1	10.7	10
Lactose	g/1	74	65
Fat	g/1	42	22
Sodium	mmol/1	6.4	5.5
Potassium	mmol/1	15	16.1
Calcium	mmol/1	8.7	6.9
Magnesium	mmol/1	1.2	1.2

References: (1) Department of Health and Social Security, 1977
(2) Gibbs, Fisher, Bhattacharya, Goddard and Baum, 1977

2.2.4 *Changes in composition with duration of lactation.* In the first few days of parturition, colostrum is secreted, whereas in established lactation 'mature' milk is produced. Changes take place in the composition of expressed breast milk over the course of lactation (Hytten, 1954). The concentration of protein (a major constituent of colostrum) decreases rapidly in the first week and then declines very slowly during the rest of the lactation period. The changes in protein concentration include changes in the proportion of nutritional proteins compared with proteins which have anti-microbial properties. About 65 per cent of the protein of human colostrum on the second day post-partum is secretory IgA and lactoferrin (McClelland, McGrath and Samson, 1978), whereas mature human milk at seven weeks after birth has less than 20 per cent of the protein as 'anti-microbial' proteins (Lönnerdal, Forsum and Hambraeus, 1976). Most of the decrease in secretory IgA concentration takes place in the first week. Lactose concentration shows a steady increase over the first month and thereafter remains unchanged; fat increases from the beginning of lactation up to the second month and then remains at the same concentration. The amounts of calcium, phosphorus, sodium, potassium and zinc decrease with the duration of lactation but magnesium concentration is almost unchanged (Jensen, Thomas, Bergmann, Filer and Fomon, 1974; Ansell, Moore and Barrie, 1977; Vaughan, Weber and Kemberling, 1979).

2.2.5 Much less is known of the changes which take place in drip breast milk as lactation proceeds. Lucas, Gibbs and Baum (1978) showed a significant decrease in the concentrations of fat, protein and sodium over the first nine months; lactose, potassium and osmolality remained constant over this time and the concentrations of calcium and magnesium increased.

2.2.6 *Significance of differences in milk composition.* Milk banks which depend mainly on drip milk from well established breast-feeding mothers at home will provide babies with a diet which is different from that provided by milk banks which depend on expressed milk from mothers who are still in hospital and in the early stages of lactation. Pooled drip breast milk will provide less energy than pooled expressed breast milk (para 2.2.3) at any

stage in lactation. Although total protein concentration is about the same in pooled drip and expressed milk, the proportion of anti-microbial protein will be greater and the proportion of nutritional protein less when the milk is collected during the early days of lactation.

2.3 Suitability of human milk for pre-term babies

2.3.1 The pre-term baby who is born early in the third trimester and who is fed human milk differs from the fetus who continues to thrive in utero and who at this stage of development is fed by a constant flow of nutrients across the placenta. The immature newborn baby has to depend on the functioning of a gastro-intestinal tract which may be physiologically unprepared to digest and absorb many nutrients, and on metabolic processes which may be incapable of utilizing some of the nutrients which have been absorbed. Thus no oral feed, even human milk, can be considered to provide optimal nutrition. It is known that some nutrients, such as protein, calcium, sodium and zinc, accumulate more rapidly in the body of the fetus in utero during the last trimester than in the body of the pre-term baby who is fed human milk (Fomon, Ziegler and Vasquez, 1977).

2.3.2 The nutritional adequacy of a food for any child can be judged by the child's state of health and rate of growth. Since the 1930s, doubts have repeatedly been expressed about the ability of human milk to provide for adequate growth of pre-term babies, particularly of those who are born very early in the third trimester (Gordon, Levine, Wheatley and Marples, 1937; Powers, 1948; Davies, 1977; Davies and Evans, 1978).

2.3.3 The relatively poor growth of pre-term babies fed human milk may in certain circumstances be associated with the smaller energy and nutritional protein content of pooled human milk (para 2.2.6). The slower growth rate has not, however, been shown to be associated with increased morbidity nor to have a detrimental effect on long-term neurological development. Neither is there any evidence that growth of pre-term infants fed on an infant milk formula is better than that of those fed on banked human milk. It is clear, however, that more attention should be paid to the variations which exist in the composition of banked human milk and in particular to the energy, protein and mineral content. The introduction of a rapid method for determining the fat content of the milk may prove helpful (Lucas, Gibbs, Lyster and Baum, 1978). There are also possibilities for modifying human milk by the addition of concentrates of some of its own constituents such as fat and protein (Lucas, Lucas, Chavin, Lyster and Baum, 1980).

2.3.4 The rapid growth of the fetus and the newborn infant creates a demand for structural lipids. These are esterified long chain fatty acids including essential fatty acids and are of particular importance in the development of the membranes of the central nervous system during the last month of a normal pregnancy. These fatty acids are produced by the

metabolism in the placenta and fetal liver of linoleic acid which is obtained from maternal blood. About 20 g of essential fatty acids have accumulated in fetal phospholipids in the full term baby (Food and Agriculture Organization, World Health Organization, 1977). If birth occurs early, the fatty acids must be supplied in milk. There is some evidence that colostrum and early milk are particularly rich in long chain fatty acids and that these decrease in concentration as lactation continues (Crawford, 1976). It is also well established that the fatty acid composition of human milk can be markedly influenced by the maternal diet (Insull, Hirsch, James and Ahrens, 1959; Department of Health and Social Security, 1980a). Banked human milk will inevitably vary in its fatty acid composition.

2.3.5 *Composition of milk from mothers of pre-term infants.* When pre-term infants are too immature to feed from the breast, mothers are encouraged to express their milk so that it can be fed to their own babies. Analysis of such milk indicates that it is different in composition from that of mothers who have been delivered at term. Initial studies show that over the first month of lactation the nitrogen content of 'pre-term milk' is about 20 per cent higher than in 'term milk' (Atkinson, Bryan and Anderson, 1978). The distribution of the total nitrogen between protein and non-protein substances in the two types of milk is similar, and there is the same pattern of decreasing concentration with time after delivery (Atkinson, Anderson and Bryan, 1980).

2.3.6 In spite of uncertainties about the nutritional adequacy of banked human milk for the feeding of pre-term babies, there is general agreement that it remains the best milk for such babies and that its unique nutritional and protective factors, even if modified by treatment after collection, are of advantage to the baby.

3. Microbiological aspects of banked human milk

3.1 Microbial content of the milk

3.1.1 Several surveys have shown, by a variety of methods, that samples of pooled human milk, even when collected under supervision, may contain large numbers of bacteria (Williamson, Hewitt, Finucane and Gamsu, 1978; McEnery and Chattopadhyay, 1978; Lucas and Roberts, 1979). Most of these bacteria are skin microflora but some are environmental micro-organisms which probably contaminate the milk during the process of collection or handling of the collected milk.

3.1.2 Banked human milk may be hazardous to babies if it contains harmful bacteria, toxins or viruses. Toxins produced by bacteria during growth, or released following their death, may also be of significance in milk. Viruses, including cytomegalovirus and sub-units of hepatitis B virus, have been identified in milk secreted by healthy carriers (Hayes, Danks, Gibas and Jack, 1972; Boxall, Flewett, Dane, Cameron, MacCallum and Lee, 1974; Linnemann and Goldberg, 1974).

3.1.3 The degree of hazard from these organisms and toxins is not yet known, but is likely to differ according to the source of the milk and the state of the baby. Considerations which apply for milk fed to a baby from his own mother may be different from those which apply to milk from a pool fed to a low birthweight sick infant (Baum, 1979b).

3.1.4 The number of micro-organisms in milk should be minimal before it is used. This may be accomplished by preventing the access of organisms to the milk, by minimizing the growth and reproduction of any micro-organisms which are present, and either by killing them or by treating the milk in some way which will reduce their numbers.

3.1.5 Milk for banking can be examined microbiologically immediately after collection and at various stages in the handling processes. Testing of milk before pooling has the advantage that donors who are unsatisfactory from the microbiological point of view (para 4.8.3.2) may be identified and reinstructed or rejected.

3.2 Methods of reducing microbial content of milk

3.2.1 Milk should not be accepted for banking from mothers who have

generalized infections or local lesions of the breast. Screening mothers for the presence of hepatitis B surface antigen (HB_sAg) is possible but, as the HB_sAg carriers do not appear to present any hazard to their own breast-fed babies (Beasley, Stevens, Shiao and Meng, 1975) and there is no evidence that hepatitis has been transmitted through banked human milk, screening of potential milk donors is not recommended at present. Screening for cytomegalovirus (CMV) is also possible but, since the virus is destroyed by heat, such identification may be unnecessary if the milk is to be heat-treated.

3.2.2 Contamination from the skin of the nipples may be reduced by attention to personal hygiene, but to clean and disinfect too vigorously may have an adverse effect on milk production by causing sore or cracked nipples which may become infected, and possibly by interfering with the normal physiology of lactation.

3.2.3 The milk should be received into a container that is as clean and free from micro-organisms as possible.

3.2.4 The growth of micro-organisms will be minimized if the milk is kept under adequate temperature control in a properly maintained domestic refrigerator or freezer. Transport and further handling of the milk should be carried out under conditions which are as hygienic as possible, and which minimize the rate of temperature increase.

3.2.5 Micro-organisms differ in their sensitivity to heat. Most organisms likely to be found in milk are reduced very greatly in number by exposure to a temperature of 62.5°C for 30 minutes (Holder pasteurization), although some strains are relatively heat-resistant. CMV is sensitive to heat but the HB_sAg particle is relatively resistant.

3.2.6 Milk that has been boiled supports the rapid growth of organisms. In contrast, milk pasteurized by the Holder method is similar to raw milk in its ability to inhibit the growth of an inoculum of *Escherichia coli* at 37°C (Gibbs, Fisher, Bhattacharya, Goddard and Baum, 1977). This suggests that some of the bacteriostatic factors in human milk are destroyed by boiling but remain largely intact after pasteurization.

3.3 Effects of heat treatment on anti-microbial constituents of banked human milk.

3.3.1 Heat treatment may damage the protective anti-microbial factors in milk. These include milk cells, immunoglobulins (predominantly secretory IgA), lysozyme, lactoperoxidase, iron-binding protein (lactoferrin) and vitamin-binding proteins. The relative clinical importance of these factors is not known. The effects of various forms of heat treatment on some of the protective factors in human milk have been studied by various workers, whose conclusions are summarized in Table 3.1. The conditions of pasteuriza-

tion, including the times and temperatures used, have varied as have the size and shape of the containers. However, most of the experiments have been conducted under Holder pasteurization conditions (para 3.2.5).

Table 3.1 The percentage survival of some protective anti-microbial factors in human milk after heat treatment at different temperatures for different periods of time (references in parenthesis)

Anti-microbial factor	Temperature in °C and time of exposure to heat in minutes					
	56° x 30′	62.5° x 30′	70° x 15′	73° x 30′	100° x 3′	100° x 15′
Immunoglobulin A	96 (1)	67–100 (1–5)	48 (1)	0 (2)	0 (4)	0 (5)
Immunoglobulin M	100 (1)	0 (1)				
Lysozyme		64–100 (1, 2, 4)	65 (1)	2 (2)	0 (4)	3 (1)
Lactoferrin		40–100 (1, 2, 5)	5 (1)	1 (2)		0 (5)
Vitamin B_{12} binding capacity		52 (1)	33 (1)			43 (1)
Folic Acid binding capacity		90 (1)	65 (1)			7 (1)

References:
(1) Ford, Law, Marshall and Reiter, 1977
(2) Evans, Ryley, Neale, Dodge and Lewarne, 1978
(3) Liebhaber, Lewiston, Asquith, Olds-Arroyo and Sunshine, 1977
(4) Gibbs, Fisher, Bhattacharya, Goddard and Baum, 1977
(5) Raptopoulou-Gigi, Marwick and McClelland, 1977.

3.3.2 *Milk cells.* It seems unlikely that any form of human milk banking can be devised which will secure the delivery to the baby of immunologically functional milk cells (Paxson and Cress, 1979; Baum, 1979c). The cells are damaged by handling procedures, even by contact with the walls of collecting or feeding bottles, as well as by temperature changes (Welsh and May, 1979).

3.3.3 *Immunoglobulins.* Many workers agree that IgA is well preserved under conditions of Holder pasteurization, but that it is progressively destroyed if the milk is subjected to a temperature of 62.5°C for longer than 30 minutes, or if the temperature is increased above 62.5°C (Ford, Law, Marshall and Reiter, 1977; Liebhaber, Lewiston, Asquith, Olds-Arroyo and Sunshine, 1977; Gibbs, Fisher, Bhattacharya, Goddard and Baum, 1977; Raptopoulou-Gigi, Marwick and McClelland, 1977; Evans, Ryley, Neale, Dodge and Lewarne, 1978). There is little immunoglobulin M in human milk and it will survive exposure to a temperature of 56°C for 30 minutes but is destroyed if kept at 62.5°C for the same length of time (Ford, Law, Marshall and Reiter, 1977).

3.3.4 *Lysozyme.* Between 60 and 100% of lysozyme is preserved by the Holder pasteurization method, but heating at 70°C or more causes the

progressive destruction of the enzyme. Only 3% of enzyme activity survives heating at 100°C (Ford, Law, Marshall and Reiter 1977).

3.3.5 *Lactoperoxidase.* Mature human milk has a lactoperoxidase concentration of one-twentieth that present in cows' milk. Up to half the activity of the enzyme in cows' milk is lost by heating at 62.5°C for 30 minutes. The effect of heat treatment on the lactoperoxidase activity of human milk has not been determined. The mode of action and importance of lactoperoxidase have recently been reviewed (Reiter, 1978) and it seems unlikely that any appreciable amount of the enzyme will be present in human milk after Holder pasteurization.

3.3.6 *Lactoferrin.* Holder pasteurization results in some loss but holding at 70°C for 15 minutes results in virtually complete destruction of lactoferrin (Ford, Law, Marshall and Reiter, 1977).

3.3.7 *Vitamin-binding proteins.* Vitamin B_{12} binding capacity is halved by heating to 62.5°C for 30 minutes. Holder pasteurization also destroys 10% of folate binding capacity. This loss is progressive as the temperature is increased so that over 90% of folate binding capacity is destroyed by heating at 100°C for 15 minutes (Ford, Law, Marshall and Reiter, 1977).

3.4 Effects of heat treatment and other procedures on the nutrient composition of milk

3.4.1 *Fat.* The fat content of milk and the efficiency of fat absorption may be affected by freezing, storage and heating. Freezing may increase the size of fat globules with a consequent reduction in surface area and thus in the available substrate for the action of lipases. The process of freeezing may also lead to adhesion of fat to the sides of the storage vessels and thereby cause a reduction in the energy content of milk actually fed to the baby. Freezing for long periods of time may lead to physical changes which cause coagulation of the milk on thawing. The magnitude of these effects in terms of energy and nutrients available to and absorbed by the baby remains to be determined.

3.4.2 Heating human milk may reduce fat absorption (Williamson, Finucane, Ellis and Gamsu, 1978). This has been attributed to losses of milk lipases which are known to be heat labile (Jenness and Patton, 1959).

3.4.3 *Vitamins.* At present there is no information about the effect of heat on the vitamin concentrations of banked human milk. Pasteurization of cows' milk has little effect on vitamins with the exception of Vitamin C which may suffer losses of up to 50% (Kon, 1961; Department of Health and Social Security, 1980a).

3.4.4 The significance of any other effects of heat treatment on nutrients has still to be determined.

3.5 Microbiological safety in relation to the preservation of protective anti-microbial factors in human milk

3.5.1 The aim of heat treatment of human milk is to make it bacteriologically 'safe', that is to say, not harmful to the infant, without at the same time causing undue destruction of protective anti-microbial factors and of nutrients.

3.5.2 For any bacterial species, there is a linear relationship between the logarithm of the number of surviving organisms at a given temperature and the length of time for which the organisms were exposed. Because the rate of thermal destruction is exponential, the point at which all the organisms in the milk are destroyed is, in theory, never reached. The time required to reduce the microbial count to a satisfactorily low figure will depend on the temperature and the number of organisms initially present in the milk.

3.5.3 A similar linear relationship between surviving activity and time at a given temperature exists for the heat inactivation of the many anti-microbial factors in milk. The rate of destruction of these factors, however, differs from that of most bacteria. Baum (1979c) has shown that, for milk IgA, the rate of destruction of the immunoglobulin is slower than that of most micro-organisms. Thus when almost all micro-organisms have been destroyed, there is still an appreciable amount of IgA left. Rates of destruction vary between the different anti-microbial factors, as they do between different micro-organisms, but present evidence indicates that Holder pasteurization, in which milk is subjected to a temperature of 62.5°C for 30 minutes, results in adequate bacteriological safety without undue destruction of anti-microbial factors. Holder pasteurization conditions were, however, defined for the commercial treatment of cows' milk, and further investigation might show that for the processing of human milk for milk banks a different combination of time and temperature would be more appropriate.

4. Organization of human milk banks

4.1 Introduction

4.1.1 Members of the Working Party do not wish to imply that existing human milk banks should alter their methods to conform to the recommendations of this report when time and experience have proved that these methods are successful. This report can only draw attention to known facts and to the methods already available, and offer suggestions which it is hoped will allow the donor to collect her milk with the minimum of inconvenience to herself and the bank to provide milk which is nutritionally adequate and microbiologically safe for infants who cannot feed from the breast. The scientific basis of human milk banking is still evolving and there is much current research on this subject.

4.1.2 A review of the methods which are at present employed for the collection and distribution of human milk has revealed considerable divergence in approach to the problems of organization. Clearly, a degree of flexibility is required to accommodate local facilities and needs and also to allow for differences in opinion about, for example, the use of untreated versus heat-treated milk (Björksten, Burman, de Château, Fredrikzon, Gothefors and Hernell, 1980; Baum, 1980).

4.1.3 If a human milk bank is to be established, the hospital concerned will have its own particular organizational problems and order of priorities. A relatively small maternity hospital or department with, for example, fewer than 2,000 births per year may not in fact establish a bank as such but be able to satisfy most of the needs of the babies in its Special Care Unit with milk donated freshly from their own mothers or other mothers in the hospital. Bigger hospitals or departments, especially those to which babies are transferred or referred from other areas, may need to consider establishing a milk bank. It is likely that in any one bank there will be more than one type of milk donated and more than one way of handling the various milks.

4.1.4 For ease of description, the problems which may arise in the organization of a milk bank will be discussed at the various stages of transferring milk from the mother to the milk bank and from the bank to the baby. This section will deal with problems associated with pooled milk for the benefit of any infant who requires the milk. The requirements for a mother who collects milk solely for the use of her own baby are discussed in section 5.

4.2 Choice of donors

Potential donors should be known to be in good health. Enquiries should be made regarding current medication. Many drugs are known to be secreted in human milk (D'Arcy, 1979; Lien, 1979) and may preclude the use of such milk.

4.3 Production of milk

4.3.1 *Drip milk.* In some women when the baby is feeding from one breast, milk will leak from the opposite breast and can be collected in sterile breast shells. The collection of drip milk is comparatively easy but the composition of the milk more closely resembles that of fore milk than hind milk and the energy and fat content is less than that of expressed breast milk (para 2.2.3).

4.3.2 *Expressed milk.* Milk can be expressed by manual massage, by a hand pump or by a mechanical pump. Such milk will usually be obtained after the baby has finished feeding and will therefore provide hind milk only and will have a larger fat and energy content (Hytten, 1954) than the expressed milk already described (para 2.2.3). When this method of collection is used there are problems in maintaining the sterility of the pumps. Pumps should be capable of being easily taken apart for thorough cleaning as otherwise substantial bacterial contamination is inevitable.

4.3.3 Mothers may use both methods and some milk banks accept a mixture of drip milk and expressed milk.

4.4 Collection of milk

4.4.1 Before milk can be accepted from a donor, the techniques of collection and the importance of cleanliness, including how to use and disinfect all the equipment, should be discussed with her. She may need to be shown how to handle pre-sterilized equipment so that it does not become contaminated.

4.4.2 Donors must be provided with a supply of equipment for disinfection. Hypochlorite is regarded as satisfactory for home use. A fresh solution should be made up daily in the container specially provided for this purpose. After disinfection, the equipment to be used for collecting the milk should be adequately drained, although small residual amounts of hypochlorite are non-toxic and may reduce the bacterial content of the milk (Lloyd Jones, Jennison and d'Souza, 1979).

4.4.3 If drip milk is to be used, collection will normally be made during each feed. Expression of breast milk may be carried out at any time though this is usually after the baby has fed. Initially mothers may express frequently to

help prevent over-distension but most women will settle into a routine of expressing every 3-4 hours. The amounts of milk obtained at any time by either method will obviously vary.

4.4.4 When milk donors are carefully screened and trained in the methods of manual expression, bacterial colony counts are low. However, because the first few millilitres (ml) of expressed milk may yield colony counts higher than in the subsequent milk, some milk banks may wish to consider discarding the first few ml before collecting the milk (Asquith and Harrod, 1979; West, Hewitt and Murphy, 1979).

4.4.5 The choice of material employed in the construction of the containers used for collecting and storing human milk is not thought to be critical under the normal operating conditions of a well run human milk bank. However, it is important that the container and its closure should be completely leak-proof, and both should be capable of retaining this property under the expected handling conditions, and across the whole temperature range to which the container may be subject.

4.5 Keeping the milk at home

4.5.1 The containers used for either individual samples or 24-hour bulk collections of milk should be either sterile or disinfected, and labelled with the donor's name, address, date of collection and a note made of any drugs being taken by the donor. Individual samples (McEnery and Chattopadhyay, 1978) should be placed immediately in the ordinary compartment of a domestic refrigerator, or added immediately to a refrigerated 24-hour bulk sample bottle which should be returned to the refrigerator as soon as possible.

4.5.2 Provided that transport to the milk bank is on a daily basis and that the milk is transported within 24 hours of collection, the milk may be kept in the domestic refrigerator near to the freezer compartment. If transport arrangements are less frequent, each completed 24-hour sample of milk should be placed in the freezer compartment of the refrigerator or in a domestic deep freeze.

4.6 Transport of milk to milk banks

4.6.1 There are several ways in which the milk may reach the milk bank, and the method of choice will depend on the local geography and facilities available. The mother or a member of the family may deliver the milk daily or every few days. In some areas it is possible for midwives or health visitors to assist in the collection of milk from the mothers. In large cities where there are several hospital units requiring milk and large numbers of scattered donors, a hospital collection and delivery service may be necessary. The filled containers are collected from the donors, fresh equipment is supplied and the

milk delivered to the hospital milk bank(s). The use of voluntary workers to help with collections may have advantages.

4.6.2 Whichever system for transportation is used, the milk should be transferred in iceboxes or large insulated picnic boxes containing icebags.

4.7 Managing the milk on reaching the bank

4.7.1 *Record Keeping.* On arrival at the milk bank, the container should be checked to see that it is properly labelled (para 4.5.1) and that labelling includes the name of the donor and the date of collection. Care must be taken to ensure that the temperature of the milk does not rise during this reception procedure, that is to say, the milk must be put into low temperature storage within minutes of arrival. When the milk is removed from storage for use, careful records must be kept of each donation, showing the donor's name, the date and method of collection and arrival date at the milk bank. To these initial records should be added later the dates of bacteriological testing, pasteurization, post-pasteurization testing, passing as 'clean' (para 4.8.4.2) and of issue, together with the results of these tests and of any estimations of fat content. Detailed records should be kept; the information becomes essential if problems arise.

4.7.2 *Storage of the milk.* If the milk arrives frozen, it should be placed in the deep freeze until it is to be tested or treated. If the milk arrives as a chilled liquid, it should either be bacteriologically tested and/or heat treated within 24 hours or frozen until it is to be tested or treated. Milk to be used within 24 hours should be kept chilled in the ordinary section of a refrigerator.

4.7.3 *Thawing of the milk.* A controlled method of thawing frozen milk is to place it in the ordinary compartment of a domestic refrigerator overnight. The temperature at which domestic refrigerators operate varies, but during thawing the temperature of the milk should not exceed 4°C. Any frozen milk remaining in the bottle after thawing will diminish the effectiveness of the heat treatment to be given to the milk, and for this reason milk intended for pasteurization should always be inspected to ensure that thawing has been thoroughly completed.

4.8 Microbiological standards for human milk

4.8.1 *Microbiological criteria*

4.8.1.1 At every stage from the donor's breast to the infant receiving the milk, careful attention should be paid to hygienic handling, the prevention of contamination and the proper temperature for storage of human milk. The anti-microbial properties of human milk cannot counteract the effects of improper handling or storage at too high a temperature.

4.8.1.2 Microbiological criteria are useful as an objective means of assessing the safety of a particular milk. Because so little is known about the effects of micro-organisms and their products in milk fed to very young infants, only arbitrary values can be given for the numerical limits for the number of organisms present. Any criteria must specify the organisms to be looked for, the volume of milk to be screened and the details of the time and temperature of incubation. Microbiological criteria, which should be decided upon only after consultation with a microbiologist, can never safeguard against the deleterious effects of subsequent unhygienic handling or poor temperature control.

4.8.1.3 The microbiological testing of every sample of milk can be extremely helpful in providing information about the hygienic standard of each individual donor and her method of milk collection, storage and transport. Faults may be identified and corrected. However, such testing imposes a large workload on microbiological services and may have to be omitted.

4.8.1.4 If each donor's collection of milk is not to be sampled and tested individually, the collections may be pooled. Microbiological testing may then be carried out on a sample of the pooled milk. This procedure, however, considerably increases the volume of milk at risk of rejection.

4.8.1.5 This report does not contain detailed instructions concerning the microbiological criteria to be adopted, or the necessary practical methodology to be employed. The microbiologist concerned with the setting up of each individual milk bank must decide what criteria are appropriate for the particular collection and handling procedures to be adopted in that bank, and what the correct methodology to operate those criteria will be. He will also know how much microbiological testing constitutes a feasible workload for his laboratory. The following paragraphs refer to criteria employed in a few well-established milk banks. The associated methodologies are described in Appendices I — III and provide an illustration of how much microbiological work may be involved in the operation of a safe milk bank. Different criteria and microbiological methods could be equally satisfactory, and this report in no way precludes their adoption. However, any criteria or microbiological methods must be judged against the absolute requirement that they allow the provision to infants of a safe milk.

4.8.2 *Untreated milk* (See Appendix I)

4.8.2.1 Milk may in certain circumstances be fed untreated. All such milk, other than that for the mother's own baby (para 5.2), should be screened microbiologically before use. There are no generally accepted microbiological criteria for the safety of such milk. Some workers have imposed on milk to be fed untreated the stringent requirements that no potential pathogens should be detected (Davidson, Poll and Roberts, 1979; Carroll, Davies, Osman and McNeish, 1979). Such pathogens were considered to include any *staphylo-*

coccus aureus, haemolytic streptococci, *Pseudomonas* species, *Proteus* species or *Streptococcus faecalis.*

4.8.2.2 Other workers (Williamson, Hewitt, Finucane and Gamsu, 1978; Hewitt, 1980) have used different criteria to indicate that human milk is suitable to be fed untreated:

a. a total aerobic mesophilic colony count of less than 2.5×10^3 colony forming units/ml (cfu/ml) with a predominance of normal skin flora;

b. a count of *Staphylococcus aureus* of less than 1×10^2 cfu/ml;

c. a 1.0 ml sample of milk should not contain Enterobacteriaceae or *Streptococcus faecalis* on enrichment culture, and

d. no other potential pathogens detectable on direct plating.

NOTE: α haemolytic streptococci may be present and are permissible if outnumbered by *Staphylococcus epidermidis (albus),* but not as the predominant organism.

4.8.2.3 Criteria used to establish the safety of untreated milk should be carefully formulated only after discussion with microbiologists who are experienced in this work and after demonstration of the effectiveness of such criteria. Although some workers suggest that pasteurization of donated milk is unnecessary (Björksten, Burman, de Château, Fredrikzon, Gothefors and Hernell, 1980), and that such milk may safely be fed untreated without bacteriological monitoring of the samples, universal adoption of such a policy for all milk banks cannot be advocated (Baum, 1980).

4.8.2.4 All milk which does not meet the microbiological criteria used to establish the safety of milk to be fed untreated to infants must be pasteurized, if it satisfies the conditions set out in section 4.8.3.

4.8.3 *Pasteurized milk* (See Appendix II)

4.8.3.1 Holder pasteurization is a reliable method of heat treatment, and will achieve a satisfactory reduction in the numbers of any pathogenic organisms present in the milk. The efficiency of a pasteurization process depends upon the initial concentration of micro-organisms (para 3.5.2). Milk should not be accepted for pasteurization if it contains large numbers of contaminating micro-organisms which may produce toxins not destroyed by heat treatment. For example, Holder pasteurization will not destroy staphylococcal enterotoxin.

4.8.3.2 The following criteria have been suggested (Baum, Fisher and Smith, 1980) as indicating that milk is unfit for pasteurization, and such milk should not be fed to infants:

a. a total aerobic mesophilic colony count of more than 1×10^6 cfu/ml;

b. a count of *Staphylococcus aureus* of more than 1 x 10^3 cfu/ml;

c. the presence of defined organisms of faecal origin, such as *Escherichia coli, Klebsiella* species and faecal streptococci in 2 microlitres of milk, at a count greater than 1 x 10^4 cfu/ml;

d. a count of group B haemolytic streptococci of more than 1 x 10^3 cfu/ml, and

e. the presence in a specified volume of milk of unusual organisms such as *Pseudomonas aeruginosa,* spore-bearing aerobes or spore-bearing anaerobes.

4.8.4 *The determination of whether milk has been adequately pasteurized* (See Appendix III).

4.8.4.1 Various criteria may be used to determine whether pasteurization of milk has been performed adequately. Some milk banks use more rigorous criteria than others, and the most appropriate criteria and methodology for any particular bank can be decided upon only after consultation with the microbiologist co-operating in running the bank.

4.8.4.2 In practice a milk bank which employs pasteurization can use the criterion that the milk should be 'clean', that is to say, growing no heat sensitive organisms following pasteurization, as indicating that the milk is fit for use. If the methodology described in Appendix IIIa is used, to describe milk as 'clean' is equivalent to a statement that it has a total aerobic mesophilic colony count of less than 5 cfu/ml (Baum, Fisher and Smith, 1980).

4.8.4.3 Such simple microbiological testing may be augmented by the use of the phosphatase test (Aschaffenburg and Mullen, 1949), which is in widespread use in the dairy industry (Appendix IIIb). The phosphatase enzyme in cows' milk is almost totally destroyed at the combination of temperature and time of heat treatment employed in Holder pasteurization. The use of this test in assessing the adequacy of the pasteurization given to human milk assumes that the behaviour of the phosphatase enzyme in human milk is the same as that in cows' milk. This may not be correct. In spite of this, the test is of proven value in some human milk banks in the monitoring of post-pasteurization milk. It is best not used as the sole test, but in association with microbiological monitoring.

4.8.4.4 More complicated microbiological criteria to determine whether or not milk has been adequately pasteurized have been used by some milk banks (Appendix IIIc). The use of such criteria involves greater effort than the performance of a simple microbiological test to ascertain whether the milk is 'clean' (para 4.8.4.2). It does, however, allow for greater discrimination, but also poses greater difficulties, in deciding whether or not an individual sample of pasteurized milk is suitable for feeding to infants. Such criteria may be used in association with the phosphatase test.

4.8.4.5 If the results of the testing of pasteurized milk indicate that the criteria have not been met, the method of collection, storage, transport and treatment of the milk must be investigated. An individual decision should be taken as to whether or not the milk is fit for use.

4.8.5 *Conclusions*

4.8.5.1 Although untreated human milk may have advantages over heat treated milk in that the protective anti-microbial constituents are preserved and the nutrients have not been affected by heat, and although the criteria for suitability of milk for feeding to infants are arbitrary, nevertheless the members of the Working Party recommend that, in newly set up milk banks, all milk should be pasteurized as a routine unless the safety of alternative methods has been established.

4.8.5.2 The Holder method of pasteurization (62.5°C for 30 minutes) is commonly used, is known to be safe and reliable, and can be recommended. Nevertheless, other appropriate combinations of time and temperature have been used for the treatment of human milk. Such alternative methods may be developed further in the future and come into wider use after their safety and practicability have been thoroughly tested.

4.8.5.3 Whatever method of pasteurization is used, it is vital that meticulous attention is paid to the details of the technique. For the Holder method these details are set out in Appendix IV.

4.9 Responsibilities for running the milk bank

4.9.1. The milk bank staff should be responsible either for sending samples of milk to the laboratory for microbiological testing or for arranging for technical staff to come to the bank for specimens. Specially designed laboratory report forms have been found to be useful. Interpretation of the results of testing will be needed and this should be made in collaboration with microbiologists and paediatricians. Milk bank staff should also be responsible for ensuring that unpasteurized and pasteurized milks are clearly labelled and separated.

4.9.2 One named member of staff should have overall responsibility for supervising the running of the human milk bank. In most cases, this would be the responsibility of the appropriate nursing or midwifery division, but in some cases it may lie with the dietetic staff. In any case, close cooperation with the microbiologist and with the paediatrician will also be necessary.

4.9.3 Information on the ways in which some established human milk banks function may be obtained from those listed in Appendix V.

4.10 Ordering milk

4.10.1 A system should be devised whereby maternity units and paediatric units place a daily order for their requirements of human milk. It is important to prevent wastage by discouraging units from over-ordering or stockpiling milk. Milk which is not wanted within 24 hours can be safely stored in the deep freeze of the human milk bank for at least 3 months. Complete thawing is essential (para 4.7.3), and this will be assisted if the capacity of the bottles used to store frozen milk is not greater than 100 ml. Once thawed, milk should be kept in the refrigerator and used as soon as possible.

4.11 Handling of human milk in hospital wards

4.11.1 The containers of human milk supplied to the ward by the milk bank should be dated, and the milk used on the ward strictly in rotation. Milk supplied frozen should be retained in frozen storage until the day before it is required, and then thawed in the ordinary compartment of a domestic refrigerator overnight. If the milk is supplied as a thawed liquid it should be kept in the domestic refrigerator.

4.11.2 All thawed milk should be used within 24 hours. The milk should not be removed from the refrigerator until immediately before it is required for warming and feeding to an infant. The bottle should not be opened until immediately before it is needed. Once the bottle is opened, every aspect of its handling and of the feeding of its contents to the baby must be carried out with the greatest possible attention to hygiene. If contamination occurs when the bottle is opened there is a risk of infection due to microbial growth if the milk is allowed to remain at room temperature for any length of time.

5. Milk provided by the mother for the use of her own baby

5.1 A mother whose baby is too small or sick to feed at the breast, or whose domestic commitments prevent her from breast feeding her baby in hospital, may well wish and should be encouraged to donate her own milk for her own baby. The milk will be collected by one of the methods described in para 4.3 with the precautions described in paras 4.4, 4.5 and 4.6 and delivered, appropriately labelled, to the bank or to the unit caring for the baby if this is in a different hospital from the bank. Such milk should be kept for the sole use of the mother's own baby.

5.2 If it is for immediate use, the milk should be kept in the ordinary compartment of a refrigerator, whether it arrives freshly expressed, as a chilled liquid or frozen. Microbiological examination and/or pasteurization are not usually considered necessary if the mother has taken the precautions already described for collecting her milk, and the milk can be fed to her baby soon after collection. Ideally all such milk should be fed within 48 hours and certainly within 72 hours of collection.

5.3 If the milk is unlikely to be fed within 48 hours, it should be stored frozen. Such stored milk should be examined microbiologically in a manner similar to that advocated for other donor milk and an individual decision taken on the need for heat treatment (para 4.8.2). If the milk is to be pasteurized, the criteria on fitness of the milk for pasteurization (para 4.8.3) and on whether the milk has been adequately pasteurized (para 4.8.4) should be satisfied.

6. Recommendations

6.1 Babies who are pre-term, recovering from neonatal surgery, at risk of atopic disease or cows' milk intolerance or are temporarily unable to breast-feed should if possible be fed on human milk (para 2.1.1).

6.2 Mothers whose babies are unable to feed at the breast should be encouraged to express milk to be fed in as fresh a condition as possible to their own babies (para 2.1.2 and 5.1).

6.3 Where there is a need to provide human milk for babies whose mothers are unable to do so, milk will come from mothers who have a supply in excess of the needs of their own infants and who are willing to collect the surplus (para 2.1.2). In order to ensure that such milk is microbiologically safe and nutritionally adequate, a human milk bank will need to be set up for the collection, processing and distribution of such milk.

6.4 Donors should be known to be in good health. Enquiries should be made about current medication (para 4.2).

6.5 At all times the milk should be protected from contamination, by careful collection and handling and scrupulous attention to hygiene (paras 4.4.1 and 4.8.1).

6.6 After collection, milk should be stored in the ordinary compartment of a domestic refrigerator for up to 24 hours, or in the freezer compartment or deep freeze (para 4.5.2). On arrival at the milk bank, milk not to be tested and/or heat-treated within 24 hours should be frozen (para 4.7.2). Milk should be properly thawed (para 4.7.3) before heat treatment, and milk whose safety for use has been established should be fed within 24 hours of thawing (para 4.10.1).

6.7 The expert involvement of a microbiologist is essential to the planning of all aspects of the operation of a human milk bank (paras 4.8.1, 4.9.1 and 4.9.2).

6.8 A microbiologist should advise on the setting and interpretation of appropriate microbiological criteria to be used to determine:

a. whether collected milk is safe for feeding without pasteurization (para 4.8.2.2);

b. whether milk is bacteriologically acceptable for pasteurization (para 4.8.3.2) and

c. whether milk has been successfully pasteurized (para 4.8.4).

6.9 Those setting up human milk banks should adopt routine pasteurization unless they have established the safety of alternative methods (para 4.8.5.1). The Holder pasteurization method is suitable (paras 3.2.5, 4.8.3.1 and 4.8.5.2).

Appendix I

An example of a practical methodology for the microbiological examination of milk to be fed to babies untreated, (para 4.8.2.2)

Source: Hewitt, 1980

Day 1

a. *Direct Plating*

One standard loopful (Exogen 0.01 ml nominal volume, nichrome loop, Code 10) of milk is plated onto a half plate of blood agar, and another onto a half plate of MacConkey agar. The plates are incubated overnight at 37°C.

b. *Enrichment*

A Pasteur pipette is used to add approximately 1 ml of milk to 5 ml of single strength MacConkey broth. This is incubated overnight at 37°C.

Day 2

a. Under good illumination the blood agar plate is examined, and the total colony count recorded. Both plates are inspected for the presence of even single colonies of potential pathogens such as lactose fermenting coliforms, β haemolytic streptococci, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Confirmatory tests may be employed if appropriate. The plates are reincubated for a further day at room temperature.

b. The MacConkey broth is sub-cultured to a half MacConkey plate, plating for separate colonies, and incubated at 37°C.

Day 3

a. From the direct platings reincubated on Day 2 record:

i. any change in the total count from the previous day;

ii. types of organisms present;

iii. the presence of *Staphylococcus aureus* (even one colony is significant).

b. From the enrichment platings incubated on Day 2 record the presence of organisms potentially of intestinal origin:

i. Enterobacteriaceae (lactose fermenting coliforms or non-lactose fermenters);

ii. *Streptococcus faecalis.*

c. If time permits, non-lactose fermenters can be confirmed as Enterobacteriaceae by:

i. Gram reaction;

ii. Oxidase test (Cowan and Steel, 1974);

iii. Hugh and Leifson Glucose Fermentation test (Cowan and Steel, 1974).

Counts per ml will be 100 x the number of colonies on the direct platings.

Appendix II

An example of a practical method for the microbiological examination of milk to ascertain whether it is fit for pasteurization (para 4.8.3.2).

Source: Baum, Fisher & Smith, 1980.

Microbiological testing may be carried out on samples of either individual collections of milk, or of pooled milk.

Day 1

Standard loopfuls of 2 microlitres of milk are placed onto 2 blood agar plates, and a further loopful of milk is plated onto a MacConkey agar plate. One blood agar plate and the MacConkey plate are incubated overnight at 37°C; the other blood agar plate is incubated anaerobically at the same temperature.

Day 2

The blood agar plates are examined, and the total colony count recorded. All 3 plates are inspected for the presence of any potential pathogens such as lactose fermenting coliforms, β haemolytic streptococci, *Staphylococcus aureus* and *Pseudomonas aeruginosa.* Confirmatory tests may be employed if appropriate. The plates may be incubated at room temperature for a further 24 hours.

Appendix III

Examples of practical methodologies which may be used to determine whether or not heat treated milk has been adequately pasteurized (para 4.8.4.1).

a. *Simple microbiological monitoring* (Baum, Fisher and Smith, 1980).

A 200 microlitre sample of post-pasteurization milk is plated, using a sterile loop and full aseptic precautions, onto 2 blood agar plates. One plate is incubated aerobically at 37°C overnight, and the other is incubated anaerobically at the same temperature. The expectation is that the milk should be 'clean', and that neither plate should grow any organisms after incubation. This is equivalent to a statement that the total mesophilic aerobic and anaerobic colony count is equal to, or less than, 5 cfu/ml.

b. *The phosphatase test of Aschaffenburg and Mullen* (1949).

The method of employing the test is precisely defined in the Milk Special Designation Regulations 1977 (Statutory Instrument, 1977).

c. *More complex microbiological testing* (Hewitt, 1980).

A possible methodology demands one sample of approximately 3 ml of milk being sent from each batch of milk pasteurized.

Day 1

Using full aseptic precautions, and a separate sterile 1 ml graduated pipette for each transfer,

i. 1 ml of milk is placed in 10 ml Lemco broth;

ii. 1 ml of milk is added to the meat layer of a Robertson's Cooked Meat medium;

(i and ii serve as aerobic and anaerobic enrichments respectively).

iii. one standard loop (0.01 ml) is plated onto blood agar for aerobic incubation.

All cultures are incubated at 37°C.

Day 2

i. Both enrichment broths are plated onto half plates of blood agar for aerobic incubation.

ii. The Robertson's Cooked Meat broth is plated onto a half plate of blood agar for anaerobic incubation.

iii. The total colony count per ml is calculated by multiplying the number of colonies on the blood agar plate by 100. Any organisms growing are identified.

Day 3

The enrichment sub-cultures are examined and the organisms identified. 'Primary' tests such as Gram, catalase and oxidase may be employed.

The milk is regarded as satisfactory if no organisms are detected. This is the normal finding. Aerobic heat sensitive non-pathogens such as *Staphylococcus epidermidis (albus)* or oxidase positive Gram negative rods (not *Pseudomonas aeruginosa*) may be identified after one or other of the 2 enrichments; this would not necessarily preclude the use of the milk. They should *not* appear on direct plating. Enterobacteriaceae and *Staphylococcus aureus* should not be present. A small proportion of samples may contain less than 100 cfu/ml of *Bacillus* species, *Clostridium* species or *Streptococcus faecalis*. The organisms present should be identified. If clostridia are found, a standard loop of milk should be plated onto blood agar and incubated anaerobically, to check that the count is less than 100 cfu/ml.

Appendix IV

Practical considerations for the pasteurization of milk

1. Whatever method of heat treatment is decided upon, for adequate Holder pasteurization the equipment must be capable of raising the temperature of the thawed milk, which is assumed to be at 0°C, to 62.5°C + or − 0.5°C and of holding the milk at that temperature for 30 minutes. The milk should be completely thawed before heating and should be heated and cooled as rapidly as possible. It is suggested that the temperature of the thawed milk should be raised to 62.5°C + or − 0.5°C within 15 minutes, and after holding, the mixed milk temperature should be reduced to 25°C within 10 minutes. The bottles should then immediately be transferred to a refrigerator.

2. Heat treatment of the milk can be controlled only if the milk is treated in special containers of standard capacity and shape so that a constant milk load is present in the pasteurizer. Bottles should be of the same type and size and always filled to the same level. Empty bottles should be filled with water. Bottles should always be loaded into the pasteurizer in the same special pattern, and immersed to the same depth so that the heating water level is at least above the level of the milk in the containers. To ensure rapid and uniform heating of the load it may be necessary for bottles to be carried on an agitated or shaken platform, and in these circumstances the bottles will need to be totally immersed beneath the water. These conditions can best be assured if the bottles are loaded in a suitable retaining crate specially designed for use with the pasteurizer.

3. Preliminary trials need to be undertaken to establish the initial temperature, heat-up times and maintained temperatures of the various parts of the load in relation to the water bath temperature as recorded by continuous temperature recorders. To carry out these trials properly, expert advice is essential. Once a master temperature record has been made for a representative load, any subsequent records made when the pasteurizer is operating routinely can be checked by comparison. Deviations in the performance of the pasteurizer can then be quickly observed and any faults remedied.

4. The temperature of the milk during pasteurization must be accurately known throughout the whole process, and, to ensure this, a correctly calibrated continous temperature recorder should be fitted to the pasteurizer water bath. Although such a purchase will involve a substantial initial financial outlay, the cost will be offset by the ability to provide an accurately

known heat treatment for every batch of milk. The operator should use the temperature recorder to ensure that the correct combination of time and temperature of heat treatment has been given to the milk. Without such a device, control of the heat process is dependent upon the efficiency and thoroughness of the individual operator, whose reliability and attention to detail cannot be checked.

5. The filling and sealing of the containers prior to pasteurization of the milk must be done as hygienically as possible. After pasteurization, the containers should not be handled whilst wet and warm, as in this state the seals are vulnerable to microbiological contamination.

6. Whatever equipment is used to pasteurize the milk, it should be capable of thorough cleaning. Water baths are a recognized source of microbial contamination, and it is essential that they should be regularly stripped down for cleaning and disinfection.

7. The containers and seals used for milk to be pasteurized must not leak. In particular, there must be no ingress of water from the pasteurizer water bath during the cooling phase. If even microscopic leakage occurs at this point, the pasteurized milk may be contaminated. Water used for cooling should, if at all possible, be taken directly from a mains supply. Mains water which has been stored in a holding tank is less satisfactory. The bacteriological quality of the water used for cooling should be checked at regular intervals. Weekly checks of the total aerobic colony count of such water should also be made and would be considered satisfactory if a colony count of less than 100 organisms/ml were obtained after incubation for 5 days at 20-22°C. Monthly checks for coliform organisms and *Pseudomonas aeruginosa* should be undertaken and these organisms should not be detectable in 100 ml of water. If there is any doubt about the bacteriological quality of the water, effective chlorination is necessary. The water should be in contact with the source of chlorine for at least 20 minutes before being used for cooling. The advice and assistance of the local Public Health Laboratory and the Water Authority are essential when problems with the bacteriological quality of cooling water are experienced or when chlorination is being considered. The local Water Authority should always be informed that mains water is being used for cooling pasteurized milk to be fed to babies.

8. Accurate records of the time and temperature of the heat treatment given to each identified batch of milk must be kept. Without such records, it is impossible to be confident that any particular batch of milk has been properly pasteurized. If any milk is found to be not 'clean' after pasteurization, such records will be an indispensable aid to the identification of any handling and processing faults which may have affected the safety of the milk.

9. After pasteurization the milk must be handled with scrupulous attention to hygiene. Micro-organisms allowed to contaminate pasteurized milk may grow rapidly because of the lack of a competitive flora.

10. Immediately after it is pasteurized, milk must be rapidly cooled, and kept under temperature control until used. It should be microbiologically tested and declared safe for feeding to infants before use and should be kept frozen until the results of testing are known.

Appendix V

Established human milk banks in Great Britain from which further information may be obtained.

BARNSLEY	District General Hospital
BIRMINGHAM	Sorrento Maternity Hospital
BRIGHTON	Royal Alexandra Hospital for Sick Children
BRISTOL	Southmead Hospital
CAMBRIDGE	Maternity Hospital
CARDIFF	St David's Hospital
CREWE	Leighton Hospital
DUNDEE	Ninewells Hospital
GLASGOW	Queen Mother's Hospital
LEICESTER	Royal Infirmary Maternity Hospital
LIVERPOOL	Alder Hey Children's Hospital Fazakerley Hospital
LONDON	Hospital for Sick Children King's College Hospital Queen Charlotte's Maternity Hospital Whipps Cross Hospital
MANCHESTER	Booth Hall Children's Hospital
OXFORD	John Radcliffe Hospital
SCUNTHORPE	General Hospital

References

American Academy of Pediatrics, 1943.
Committee on Mother's Milk. Recommended standards for the operation of mother's milk bureaus.
Journal of Pediatrics, **23,** 112.

Ansell, C., Moore, A. and Barrie, H., 1977.
Electrolyte and pH changes in Human Milk.
Pediatric Research, **11,** 1177-1179.

Aschaffenburg, R. and Mullen, J. E. C., 1949
A rapid and simple phosphatase test for milk.
Journal of Dairy Research, **16,** 58-67.

Asquith, M. T. and Harrod, J. R., 1979.
Reduction of bacterial contamination in banked human milk.
Journal of Pediatrics, **95,** 993-994.

Atkinson, S. A., Bryan, M. H. and Anderson, G. H., 1978.
Human milk: difference in nitrogen concentration in milk from mothers of term and premature infants.
Journal of Pediatrics, **93,** 67-69.

Atkinson, S. A., Anderson, G. H. and Bryan, M. H., 1980.
Human milk: comparison of the nitrogen composition in milk from mothers of premature and full-term infants.
American Journal of Clinical Nutrition, **33,** 811-815.

Baum, J. D., 1979a.
Development of Human Milk Banks.
Midwife, Health Visitor and Community Nurse, **15,** 126-129.

Baum, J. D., 1979b.
Raw breast milk for babies on neonatal units.
Lancet, **ii,** 898.

Baum, J. D., 1979c.
The effects of pasteurisation on immune factors in human milk.
In: *Nutrition and metabolism of the fetus and infant,* ed. K. H. A. Visser, pp 273-283.
The Hague, Martinus Nijhoff.

Baum, J. D., 1980.
Collecting and banking human milk: to heat or not to heat.
British Medical Journal, **2,** 1066-1067.

Beaum, J. D., Fisher, C. and Smith, A., 1980.
A Guide to Human Milk Banking.
Basingstoke, Vickers Medical.

Beasley, R. P., Stevens, C. E., Shiao, I. S. and Meng, H. C., 1975.
Evidence against breast-feeding as a mechanism for vertical transmission of hepatitis B.
Lancet, **ii,** 740-743.

Björksten, B., Burman, L. G., de Château, P., Fredrikzon, B., Gothefors, L. and Hernell, O., 1980.
Collecting and banking human milk: To heat or not to heat
British Medical Journal, **2,** 765-769.

Boxall, E. H., Flewett, T. H., Dane, D. S., Cameron, C. H., MacCallum F. O. and Lee, T. W., 1974.
Hepatitis B surface antigen in breast milk.
Lancet, **ii,** 1007-1008.

Carroll, L., Davies D. P., Osman, M. and McNeish, A. S., 1979.
Bacteriological criteria for feeding raw breast milk to babies on neonatal units.
Lancet, **ii,** 732-733.

Cowan, S. T. and Steel, K. J., 1974.
Identification of medical bacteria, 2nd edition.
Cambridge University Press.

Crawford, M. A., 1976.
Milk lipids and their variability.
Current Medical Research and Opinions, **4,** Supplement 1-33.

D'Arcy, P. F., 1979.
Drugs in milk
In: *Iatrogenic diseases* 2nd edn, ed. P. F. D'Arcy and J. P. Griffin pp 425-435.
Oxford University Press.

Davidson, D. C., Poll, R. A. and Roberts, C., 1979.
Bacteriological monitoring of unheated human milk.
Archives of Disease in Childhood, **54,** 760-764.

Davies, D. P., 1977.
Adequacy of expressed breast milk for early growth of pre-term infants.
Archives of Disease in Childhood, **52,** 296-301.

Davies, D. P. and Evans, T. J., 1978.
Nutrition and early growth of pre-term infants.
Early Human Development, **2,** 383-392.

Department of Health and Social Security, 1974.
Report on Health and Social Subjects No. 9.
Present-day practice in infant feeding.
London, HMSO

Department of Health and Social Security, 1977.
Report on Health and Social Subjects No. 12.
The composition of mature human milk.
London, HMSO

Department of Health and Social Security, 1980a.
Report on Health and Social Subjects No. 18.
Artificial feeds for the young infant.
London, HMSO

Department of Health and Social Security 1980b.
Report on Health and Social Subjects No. 20.
Present day practice in infant feeding: 1980.
London, HMSO

Evans, T. J., Ryley, H. C., Neale, L. M., Dodge, J. A. and Lewarne, V. M., 1978.
Effect of storage and heat on anti-microbial proteins in human milk.
Archives of Disease in Childhood, **53,** 239-241.

Food and Agriculture Organization, World Health Organization, 1977.
Dietary fats and oils in human nutrition.
Report of an expert consultation.
Rome, F.A.O.

Fomon, S. J., Ziegler, E. E. and Vasquez, H. D., 1977.
Human milk and the small premature infant.
American Journal of Diseases of Children, **131,** 463-467.

Ford, J. E., Law B. A., Marshall, V. M. E. and Reiter, B., 1977.
Influence of the treatment of human milk on some of its protective constituents.
Journal of Pediatrics, **90,** 29-35.

Gibbs, J. H., Fisher, C., Bhattacharya, S., Goddard, P. and Baum, J. D., 1977.
Drip breast milk: its composition, collection and pasteurization.
Early Human Development, **1,** 227-245.

Gordon, H. H., Levine, S. Z., Wheatley, M. A. and Marples, E. 1937.
Respiratory metabolism in infancy and childhood, The nitrogen metabolism in premature infants — comparative studies of human milk and cows' milk.
American Journal of Diseases of Children, **54,** 1030-1047.

Hayes, K., Danks, D. M., Gibas, H. and Jack, I., 1972.
Cytomegalovirus in human milk.
New England Journal of Medicine, **287,** 177-178.

Hewitt, J. H., 1980.
Personal communication.
Public Health Laboratory, Dulwich Hospital, London, SE22 8QF.

Hytten, F. E., 1954.
Clinical and chemical studies in human lactation.
British Medical Journal, **1,** 175-182 and 249-255.

Insull, W., Hirsch, J., James, A. T. and Ahrens, E. G., 1959.
The fatty acids in human milk II Alteration produced by manipulation of caloric balance and exchange of dietary fats.
Journal of Clinical Investigation, **38,** 443-450.

Jenness, R. and Patton, S., 1959.
Milk enzymes. Chapter 6 in *Principles in Dairy Chemistry* pp 182-203.
New York: J. Wiley and Sons Inc.

Jensen, R. L., Thomas, L. N., Bergmann. K. E., Filer L. J., Jnr. and Fomon, S. J., 1974.
In: *Infant Nutrition* 2nd edn. ed. S. J. Fomon, pp 359-407.
W. B. Saunders, Philadelphia, PA.

Kon, S. K., 1961.
Nutritional effects on milk of chemical additives and processing.
Federation Proceedings, **20,** 209-216.

Liebhaber, M., Lewiston, N. J., Asquith, T. T., Olds-Arroyo, L. and Sunshine, P., 1977.
Alterations of lymphocytes and antibody content of human milk after processing.
Journal of Pediatrics, **91,** 897-900.

Lien, E. J., 1979.
The excretion of drugs in milk, a survey.
Journal of Clinical Pharmacy, **4,** 133-144.

Linnemann, C. C. and Goldberg, S., 1974.
HB Ag in breast milk.
Lancet, **ii,** 155.

Lloyd Jones, C., Jennison, R. F., and d'Souza, S. W., 1979.
Bacterial contamination of expressed breast milk.
British Medical Journal, **2,** 1320-1322.

Lönnerdal, B., Forsum, E. and Hambraeus, L., 1976.
A longitudinal study of the protein, nitrogen and lactose contents of human milk from Swedish well-nourished mothers.
American Journal of Clinical Nutrition, **29,** 1127-1133.

Lucas, A., Gibbs, J. A. H. and Baum, J. D., 1978.
The biology of human drip breast milk.
Early Human Development, **2,** 351-361.

Lucas, A., Gibbs, J. H., Lyster, R. L. J. and Baum, J. D., 1978.
The Creamatocrit: simple clinical technique for estimating fat concentration and energy value of human milk.
British Medical Journal, **1,** 1018-1020.

Lucas, A., Lucas, P. J., Chavin, S. I., Lyster, R. L. J. and Baum, J. D., 1980.
A human milk formula.
Early Human Development, **4,** 15-21.

Lucas, A. and Roberts, C. D., 1979.
Bacteriological quality control in human milk banking.
British Medical Journal, **1,** 80-82.

McClelland, D. B. L., McGrath, J. and Samson, R. R., 1978.
Antimicrobial factors in human milk. Studies of concentration and transfer to the infant during the early stages of lactation.
Acta Paediatrica Scandinavica, Supplement **271,** 1-20.

McEnery, G., and Chattopadhyay, B., 1978.
Human milk bank in a District General Hospital.
British Medical Journal, **2,** 794-796.

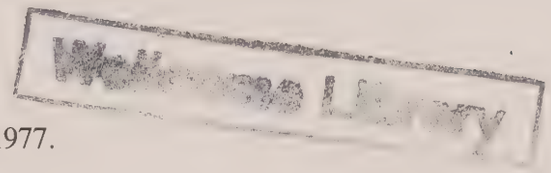
Paxson, C. L. and Cress, C. C., 1979.
Survival of human milk leukocytes.
Journal of Pediatrics, **94,** 61-63.

Powers, G. F., 1948.
Some observations on feeding of premature infants based on 20 years' experience at New Haven Hospital.
Pediatrics, **1,** 145-158.

Raptopoulou-Gigi, M., Marwick, K. and McClelland, D. B. L., 1977.
Antimicrobial proteins in sterilised human milk.
British Medical Journal, **1,** 12-14.

Reiter, B., 1978.
Review of the progress of Dairy Science: antimicrobial systems in milk.
Journal of Dairy Research, **45,** 131-147.

Siimes, M. A., and Hallman, N., 1978.
A perspective on human milk banking, 1978.
Journal of Pediatrics, **93,** 992-993.



Statutory Instrument, 1977.
No. 1033 The Milk Special Designation Regulations 1977.
London, HMSO

Vaughan, L. A., Weber, C. W. and Kemberling, S. R., 1979.
Longitudinal changes in the mineral content of human milk.
American Journal of Clinical Nutrition, **32,** 2301-2306.

Welsh, J. K. and May, J. T., 1978.
Anti-infective properties of breast milk,
Journal of Pediatrics, **93,** 1-9.

West, P. A., Hewitt, J. H. and Murphy, O. M., 1979.
The influence of methods of collection and storage on the bacteriology of human milk.
Journal of Applied Bacteriology, **46,** 269-277.

Williamson, S., Finucane, E., Ellis, H. and Gamsu, H. R., 1978.
Effect of heat treatment of human milk on absorption of nitrogen, fat, sodium, calcium and phosphorus by pre-term infants.
Archives of Disease in Childhood, **53,** 553-563.

Williamson, S., Hewitt, J. H., Finucane, E. and Gamsu, H. R., 1978.
Organisation of bank of raw and pasteurised human milk for neonatal intensive care.
British Medical Journal, **1,** 393-396.

Printed in England for Her Majesty's Stationery office by Linneys of Mansfield
Dd 718949 C20 6/81

HER MAJESTY'S STATIONERY OFFICE

Government Bookshops

49 High Holborn, London WC1V 6HB
13a Castle Street, Edinburgh EH2 3AR
41 The Hayes, Cardiff CF1 1JW
Brazennose Street, Manchester M60 8AS
Southey House, Wine Street, Bristol BS1 2BQ
258 Broad Street, Birmingham B1 2HE
80 Chichester Street, Belfast BT1 4JT

Government publications are also available through booksellers

ISBN 0 11 320765 4